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# HAZARD EVALUATION DIVISION

# STANDARD EVALUATION PROCEDURE

# HYDROLYSIS STUDIES

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16. Abstract (Limit: 200 words)

The Standard Evaluation Procedure for the Hydrolysis Studies is a guidance document primarily intended for Agency reviewers and the regulated industry who evaluate data specified in 40 CFR Part 158.124. The SEP is also intended to provide information to the general public indicating how the Agency evaluates these types of studies. As such, it is designed to supplement Subdivision N of the Pesticide Assessment Guidelines - Chemistry: Environmental Fate.

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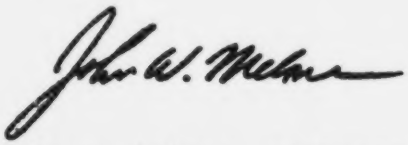
## STANDARD EVALUATION PROCEDURE

## PREAMBLE

This Standard Evaluation Procedure (SEP) is one of a set of guidance documents which explain the procedures used to evaluate environmental and human health effects data submitted to the Office of Pesticide Programs. The SEPs are designed to ensure comprehensive and consistent treatment of major scientific topics in these reviews and to provide interpretive policy guidance where appropriate. The Standard Evaluation Procedures will be used in conjunction with the appropriate Pesticide Assessment Guidelines and other Agency Guidelines. While the documents were developed to explain specifically the principles of scientific evaluation within the Office of Pesticide Programs, they may also be used by other offices in the Agency in the evaluation of studies and scientific data. The Standard Evaluation Procedures will also serve as valuable internal reference documents and will inform the public and regulated community of important considerations in the evaluation of test data for determining chemical hazards. I believe the SEPs will improve both the quality of science within EPA and, in conjunction with the Pesticide Assessment Guidelines, will lead to more effective use of both public and private resources.



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# HYDROLYSIS STUDIES

## I. INTRODUCTION

### A. Objective

This Standard Evaluation Procedure (SEP) is to be used as an aid for data reviewers in the Exposure Assessment Branch while reviewing hydrolysis studies submitted by registrants in support of pesticide registration.

40 CFR Part §158.130 requires hydrolysis studies to be conducted with each pesticide active ingredient intended for use outdoors. The section in the Subdivision N Guidelines which describes this study and provides sample protocol is 161-2. Section 160-5 of the Guidelines gives general guidance on the reporting of data.

### B. General Theory

Since outdoor-use pesticides can be applied directly to aquatic systems or can enter aquatic systems via leaching or runoff after terrestrial application, knowledge of the hydrolytic fate of pesticides is critical to understanding the overall fate of pesticides in the environment.

Hydrolysis refers to the reaction of a compound with water during which bonds in the compound are broken and a net exchange between some group X on the compound with OH of water at the reaction center occurs. For background information and theory on the hydrolysis reaction, refer to Appendix A.

## II. THE SUBMITTED STUDY

### A. Purpose

The hydrolysis study is to be designed and conducted in such a way as to reliably provide the rate of hydrolysis of the parent compound, rates of formation and decline of hydrolysis products, and the identity of the hydrolysis products.

### B. Study Design

The submitted hydrolysis study should provide sufficient information on experimental design, the test substance and the experimental procedures. For a list of the information to be included in the submitted hydrolysis study, refer to Appendix B.

## III. THE EVALUATION PROCESS

### A. Determine the Need for the Study

For all data submitted as part of a registration action, the reviewer is to first determine whether the data is needed in support of the proposed use. If an initial look at the study reveals that the data supplied is not relevant and not needed to support the proposed registration action, then the reviewer should only mention in the review that the particular study was submitted but was not reviewed in detail and why.

Hydrolysis data is needed for all outdoor use pesticides; therefore, the hydrolysis study will always be reviewed in detail except in cases where the submitted study provides only hydrolysis information already known from the earlier review of other hydrolysis studies.

### B. Read the Report

Read the hydrolysis report keeping the following broad issues in mind: (1) Are the goals appropriate, (2) Was the study conducted in a scientifically sound manner to meet those goals, (3) Were the experimental design, test substance and experimental procedures adequately described and, (4) Are there data gaps that impede the review process or invalidate the study?

When reading the study, the reviewer should note whether all necessary descriptive information has been supplied in the report. Some descriptive information is so critical to the review process, such as the temperature at which the study was run, that its absence can result in not allowing the review to proceed while the absence of some information, such as loss of a single sample, will only minimally affect the review process.

### C. Prepare the Data Evaluation Record

The reviewer should now prepare the Data Evaluation Record (DER) according to the Standard Format for Preparation of Environmental Fate Reviews. Appendices B-D should be used in this process.

#### 1. Write the Technical Evaluation

In the DER of the study, the reviewer is to record (1) whether the submitted study reliably defines the rate of hydrolysis of the a.i., (2) the rates of formation and decline of the hydrolysis products and (3) the identity of the hydrolysis products.

#### 2. Determine Study Acceptability

From the scientific point of view, the reviewer now determines if the study satisfies the hydrolysis data requirement in light

of the proposed use.

3. Preparations for Making the Final Regulatory Determination

Based on the scientific results of the hydrolysis study, the reviewer now makes a statement on the possible impact the pesticide could have on the environment such as in ground water and/or surface water due to its hydrolytic persistence. This statement will be considered with similar statements from the review of the other environmental fate studies in making the final regulatory determination.

4. Determine Need for Deferral/Referral to other HED Branches

If the parent compound or any of its degradation products are found to be stable to hydrolysis, then it may impact on ground water and/or aquatic organisms. Referral of this information and other information relating to impact on ground water should be made to the appropriate branches in HED.

## APPENDIX A

## GENERAL THEORY AND REFERENCES FOR EVALUATING HYDROLYSIS STUDIES

In the hydrolysis of organic pesticides, a carbon-oxygen bond is often broken although carbon bonds with nitrogen, sulfur, and halogens can also be broken during hydrolysis.[1] Also, phosphorus-oxygen and phosphorus-sulfur bonds are susceptible to hydrolysis.[2]

Pesticides and water can react through several different mechanisms; however, the physico-chemical hydrolysis reactions that occur under conditions typically found in the environment (i.e., pH 5-9 and temperatures of 15-35°C) are usually either the substitution nucleophilic ($S_N1$ and $S_N2$) reactions[3] or ester hydrolysis.[4] In addition, an elimination ($E_2$) mechanism has been found to be responsible for the hydrolysis of dibromochloropropane (DBCP) under environmental-like conditions.[5] For an in-depth discussion of these mechanisms and more information on hydrolysis, the reviewer is referred to the excellent texts available.[6, 7, 8]

APPENDIX B

INFORMATION SUBMITTED AS PART OF THE HYDROLYSIS STUDY

The goals of the experiment.

Chemical name, common name, trade name, company designations and structures of both the a.i. and the hydrolysis products.

Water solubility of the a.i..

Source and purity of the a.i..

Site of radiolabeling (if applicable).

Source and purity of the water.

Concentration of a.i. at which the study was conducted.

Buffer solutions used and how they were prepared.

Description of experimental equipment (glassware, analytical equipment, etc.).

Complete description of the analytical procedure.

Name and signature, title, organization, address and telephone number of the persons responsible for all aspects of the laboratory procedures and analytical work.

The title, date of development and source of the analytical method (company developed, taken from the literature, etc.)

A description of the principles of the analytical method.

A copy of the method containing all steps of the procedure in detail. Especially describe those steps involving special precautions (to avoid safety or health hazards, etc.; explain). Describe any modifications made to the method.

Extraction efficiency.

Instrumentation (make/model, type/specificity of detectors, column packing materials, carrier gas, flow rates, temperatures, limit of detection and sensitivity, calibration procedures, etc.).

Interferences (if any).

Confirmatory techniques used.

Statistical treatment of data.

Other appropriate and relevant information needed to provide a thorough and complete description of the analytical methodology and the means of determining the results of the study.

Conditions of test solution (temperature, sterility, pH and darkness).

Method for adding the a.i. to the buffer solution.

Means of sterilization.

Means and technique of sampling and handling of the sample.

Storage of samples (if applicable).

Tables of results (raw data, sample calculations of concentration of analytes, correction factors applied, etc.).

Rate of hydrolysis of the a.i., rates of formation and decline of hydrolysis products and the identity of the hydrolysis products.

Cosolvent (if applicable).

Quality assurance - A complete description of the measures taken to ensure the integrity of the study such as logbooks, record-keeping procedures, sample chromatograms, sample coding, skilled personnel, use of high quality glassware/compounds/solvents, calibration and maintenance of instruments, etc.).

Contact for questions from the reviewer.

Raw data, sample chromatograms and sample calculations on how the hydrolysis rates were derived and how the hydrolysis products were identified. Also, method validation and recovery data.

Special problems with the study such as adhesion of a.i. to the test equipment, a.i. insoluble in water, emulsion formed, buffer catalysis, etc..

APPENDIX C

CONSIDERATIONS IN REVIEWING HYDROLYSIS STUDIES

Were the stated goals of the study appropriate and clearly defined? Was the study conducted in a scientifically sound manner to accomplish those goals?

Has the name and structure of the a.i. been provided?

Did the starting concentration exceed the water solubility?

Was the starting concentration too high?

Were there impurities in the a.i. that would interfere with the analytical method?

Was the site of radiolabeling in a stable portion of the a.i.? Was an appropriate isotope used?

Were the buffer solutions adequately described?

Did the pH of the buffer solutions change during the study?

Were sterile conditions maintained? Could microbial degradation affect the study?

Was the analytical procedure adequately described? Were raw data, sample chromatograms and sample calculations provided? Were the statistics verifiable?

Was the study conducted at an appropriate temperature and at suitable pHs? Was the temperature maintained? Did the pH change during the experiment? Does the use pattern dictate that the study be run under conditions outside of those recommended by the Subdivision N Guidelines (Refer to Appendix D)?

If the study was conducted while exposed to light, does photolysis contribute to degradation?

If cosolvent(s) were used, did they affect the degradation rate? Will another study be needed using less cosolvent to determine this?

Were there buffer catalysis effects?

Was there a good mass balance? If not, was the loss of material accounted for? Is the study invalid due to unaccounted for material?

Was the study conducted long enough?

Were samples taken frequently enough to establish the kinetics? Were replicates taken? Was the study of sufficient duration (Refer to Appendix D)?

Were samples stored between the time of sampling and analysis? How long? Were the conditions of storage described? Are the a.i. and its hydrolysis products stable under the storage conditions? If stored, how were the samples prepared for analysis upon removal from storage?

Was the test solution sampled immediately after addition of the a.i.?

Were appropriate solvents used for extraction/partitioning?

Is the study of such low quality or are there so many questions that you would recommend there be a lab audit?

Were the reported method sensitivity and limit of detection appropriate?

Were the kinetics of the study determined correctly?

Are the results of the study supported by the results of similar studies? Refuted by the results of similar studies?

Would residues of the a.i. and/or its hydrolysis products persist in ground water?

Was the study conducted with good quality control procedures? If applicable, determine if additional hydrolysis data are needed (such as a long-term hydrolysis study) even though the data requirement may be satisfied. Does the hydrolytic fate of the compound pose special problems or questions affecting other environmental fate studies or studies of importance to other HED branches? (For example, does a significant hydrolysis product form which should be highlighted in the review and perhaps be referred to the Toxicology or Ecological Effects branches?) Do data submitted as part of other environmental fate studies (such as the dark control of the photolysis study) support or refute the results of the hydrolysis study? Should a time limitation be imposed for submitting additional hydrolysis data?

Does the study support the proposed use?

See Appendix D for an explanation of the significance of some of the test parameters influencing hydrolysis.

APPENDIX D

TEST PARAMETERS INFLUENCING HYDROLYSIS

pH, Temperature, Light Conditions, and Initial Concentration

The Agency has received and reviewed pesticide hydrolysis studies at every pH between 1 and 14 and at temperatures between 5 and 100°C. However, applied pesticides usually do not encounter a pH outside the range of 5-9. Likewise, temperatures during the time of use of most pesticides do not usually vary outside of the 15-35°C range. Therefore, a hydrolysis study conducted at 25°C and pHs of 5, 7, and 9 would, in most cases, be adequate to describe the hydrolytic fate of the pesticide under ordinary use conditions. The temperature of the reaction should be precisely controlled since an uncertainty of ± 1°C in temperature roughly corresponds to a change of ± 10% in the rate ($k_T$).[3] Environmental temperatures of surface aqueous systems vary much more than 1°C on a monthly or even a daily basis. However, duplicating this natural fluctuation in temperature in the lab would not be practical. On the other hand, the temperature of deeper ground water has been found to be relatively constant.[9]

Some researchers prefer to conduct hydrolysis at temperatures between 50 and 80°C to speed up the reaction and then determine the rate constant at 25°C using the Arrhenius equation:

$$k = Ae^{-E_A/RT}$$

where: k is the rate constant ($time^{-1}$);

$E_A$ is the Arrhenius activation energy (kcal/mol);

R is the gas constant (1.987 cal/deg·mol);

T is the temperature (°K); and

A is a pre-exponential factor with the same units as k.

However, it is probably prudent to regard rate data obtained by extrapolation as order-of-magnitude estimates[3] and to request that hydrolysis studies be conducted at 25°C.

There are some pesticide use conditions, both agricultural and non-agricultural, that would result in pesticide usage out-

side the pH and/or temperature ranges noted above. For example, pesticides may be used in acidic environments (e.g., azaleas whose optimum soil pH is as low as 4.5,[10] highbush blueberries which grow in soils with pH as low as 4.0,[11] paper mills with pHs of 4.3 and 11.0,[12] and in mosquito breeding areas where the pH may be 3.6[13]) or at temperatures significantly above or below 25°C (e.g., paper mills and oil production fields where pesticides are added to solutions of temperatures 44-63°C[12] and 0-76°C,[14] respectively). Use of pesticides under these special situations should be supported with hydrolysis experiments conducted under these unique conditions of pH and temperature.

Since photolysis of organic compounds can occur under normal laboratory lighting conditions[15], the hydrolysis study should be conducted in darkness thereby eliminating photochemical mechanisms of degradation.

It is also important to periodically check the pH of the buffered test solution to be sure the desired pH is being maintained.

The hydrolysis study should be conducted at a starting concentration approximating the maximum concentration at which the pesticide might be expected to be found in the aquatic environment (although the Guidelines[16] allow a maximum starting concentration of 250 ppm). This is usually around 100 parts per billion for ground water contaminants[17] and for compounds partitioning into the soil water after terrestrial application to 1-20 parts per million for compounds applied to aquatic surface systems for the control of aquatic pests. Therefore, typical starting concentrations are 0.1-20 parts per million.

Levels of some pesticides may be found in aquatic systems at concentrations much less than the starting concentration of the pesticide in the hydrolysis study and there may be concern that the hydrolysis data may, therefore, not be indicative of hydrolysis that would occur in the environment. However, it has been generally observed that the rate of aqueous hydrolysis of organic compounds is first-order in the concentration of the solute and the rate of disappearance of the solute is directly proportional to its concentration,[3] as expressed by the equation:

$$\frac{-d[RX]}{dt} = k_T[RX]$$

where: [RX] = concentration of the solute; and

$k_T$ = the hydrolysis rate constant.

Since rate processes found to be simple at high concentrations remain simple at lower starting concentrations,[18] conclu-

sions derived from hydrolysis studies conducted at concentrations recommended by the Guidelines[16] should be valid for hydrolysis occurring at the lower expected environmental concentrations.

However, there have been cases where different rates of hydrolysis have been observed for the same compound with the only variable being the starting concentration. Such differences in rate might be due to adsorption onto the surface of the reaction flask or to catalysis effects[19] resulting from the necessity to use a higher buffer concentration with a high starting concentration of the parent material.

The reviewer should verify that the reported inital concentration does not exceed its water solubility.

Radiolabeling

Use of a radiolabeled pesticide provides easier accountability of material balance and an easier identification of degradation products than use of non-radiolabeled starting material. Generally, lower starting concentrations can be used due to the higher sensitivity of radio detectors over conventional analytical methods.

Carbon atoms are the most convenient atoms to tag in the starting material due to the 5,730 year half-life[20] and relative ease of handling of $^{14}C$ material. However, some aldicarb studies using $^{35}S$, which has an 88 day half-life,[20] have been successfully done.[21] Although many pesticides contain chlorine, and $^{36}Cl$ has a $3.1 \times 10^5$ year half-life,[20] use of $^{36}Cl$ to radiolabel pesticides is not done due to its high expense and the additional safety procedures needed.[22] Although $^{32}P$ has been used for metabolism studies of organo-phosphorus pesticides, it is generally not used for hydrolysis studies due to its relatively short half-life of 14.3 days.[20] Tritium ($^3H$), with a half-life of 12.3 years,[21] has been used successfully to tag pesticides for use in environmental fate studies. However, the registrant must show that proton exchange will not interfere with the experiment.

Radiolabeling should be done in a stable position of the molecule. Tagging a carbon that would be rapidly released as $^{14}CO_2$ would not be appropriate. Also, if the molecule breaks into two or more significant fragments, it may be necessary to label the parent compound in more than one site so that the hydrolytic fate of each fragment may be followed.

Mass Balance

All (> 95%) of the radioactivity used in the experiment should be accounted for to ensure that all the hydrolysis products have been quantified and that the fate of all of the starting material is understood. The reviewer must be able to ascertain that the

data on the mass balance are adequate. Traps to monitor possible production of volatile hydrolysis products must be used. Also, binding of pesticide to the surface of the test equipment should be considered. This binding can unexpectedly occur resulting in what would appear to be unaccounted mass thus invalidating the study.

Duration/Sampling

Sampling must be conducted for a period of time sufficient to define the rate of decline of the pesticide as well as the rate of appearance and decline of the degradation products. It is desirable that samples be taken over two half-lives to ensure that any deviations from the presumed first-order linearity are detectable.[3]

Since the Agency frequently uses the hydrolysis reaction rate with models for estimation of a pesticide's environmental concentration, accurate rate data are needed.[19] It is recognized that environmental variables which can affect the hydrolysis rate vary dramatically and this fact tempers the need for highly accurate rate data. Therefore, a sampling regimen which approaches the following and includes sampling immediately after addition of the pesticide to the buffer solution would provide sufficient precision.

> "Take a minimum of six regularly spaced, duplicate samples all taken between 20% and 70% hydrolysis with ± 4% precision. When only 20-30% hydrolysis occurs in the first several weeks, then 15-20 samples should be taken between 10-30% hydrolysis."[23]

A study duration of 30 days is usually sufficient to establish whether a compound will be resistant to hydrolysis. As a practical matter, if less than about 10% of the pesticide hydrolyzes within 30 days, the pesticide will be considered to be hydrolytically stable.

Generally, hydrolysis studies of 30 days duration will suffice, since dilution in the environment is likely to reduce any concerns by that time. However, in cases in which dilution is not likely to occur at a significant rate; e.g., ditch bank empoundments, ponds, ground water, etc., consideration must be given to experiments of longer duration.

Cosolvents

The solvent composition chosen can affect both the rate of the hydrolysis reaction and the products formed.[24] Use of a 100% aqueous system is ideal but a cosolvent concentration of $\leq$ 1% (of the common organic solvents) will not significantly

impact on the reaction. Preferably, cosolvents should be used only when absolutely necessary to solubilize water-insoluble pesticides to a starting concentration sufficient to allow the study to proceed.

Buffers and Catalysis Effects

Buffers should be used at the lowest concentration possible due to possible catalysis effects. In general base catalysis, the basic component of the buffer attracts a proton from water molecules, polarizing the water molecules thereby increasing their nucleophilicity and resulting in more hydrolysis.[25] In general acid catalysis, the acidic component of the buffer attracts the leaving anion of the pesticide, thereby polarizing the pesticide and causing greater attraction for hydroxyl groups at the partial positive site.

To minimize buffer effects, it is recommended that borate or acetate buffers rather than phosphate buffers be used whenever possible.[18] Also, since the starting concentration of pesticide is less than $10^{-4}$M, the buffers may be used at 0.01M concentrations to keep the pH constant, concentrations which largely reduce the likelihood of buffer effects.[18]

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